

Shrinkage of cutaneous specimens: formalin or other factors involved?

Background: Shrinkage of cutaneous tissue during processing is a source of controversy. This study was designed to prospectively determine tissue shrinkage at two intervals: 1 min after excision and after 24 to 48 h of formalin fixation. Secondly, gender, age, site, prior biopsy scar and solar elastosis were evaluated with respect to shrinkage.

Methods: Ninety-seven cutaneous specimens were measured prior to excision, 1 min after removal and after 24 to 48 h of formalin fixation. Width of prior biopsy scar, damage to elastic fibers and solar elastosis were subjectively quantified.

Results: Significant tissue shrinkage occurred immediately after excision, prior to formalin fixation. Mean shrinkage (95% confidence interval): length $20.66\% \pm 2.15\%$ and width $11.79\% \pm 2.35\%$. Range of shrinkage: length 0 to 41.18% and width -18.75% (indicating expansion) to 37.50%. Patient age was significant; shrinkage decreased 0.3% per year of increasing age. Site was less significant; trunk excisions measured 5% greater shrinkage than head/neck excisions. As solar elastosis increased, shrinkage decreased.

Conclusions: Cutaneous tissue shrinkage following excision is primarily because of intrinsic tissue contractility. Increasing patient age and solar elastosis correlate with less shrinkage. The clinicians and dermatopathologists must be cognizant of the expected shrinkage of submitted specimens for settling discrepancies within the medical record.

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Shrinkage of visceral specimens induced by processing has been reported^{1–7} however, studies utilizing cutaneous specimens are lacking. A few studies have examined cutaneous tissue shrinkage with conflicting results;^{8–11} however, most prior studies have not emphasized the importance of shrinkage as a factor in settling discrepancies in the medical record. Herein, we report a prospective evaluation of 97 excisional specimens with the purpose of determining the amount of tissue shrinkage and the factors that are contributory to the shrinkage during tissue processing. Patient's age, site and gender were determined to be contributory or non-contributory to the shrinkage, and outliers (those with the most and least shrinkage)

were further studied with elastic stains, measures of solar elastosis and scar width to determine if these additional factors were explanatory in the extremes of shrinkage noted.

Materials and methods

Ninety-seven patients (59 males, 38 females; mean age 53.6 years) having 97 lesions excised were prospectively studied over a period of 4 months. Sclerotic or infiltrative diseases and nodular tumors were excluded as these histological subtypes were presumed by the authors to be less likely to exhibit normal contractile properties. Most specimens were biopsy-proven

Kerns et al.

cutaneous carcinoma; so, a small biopsy scar was part of the excisional specimen. Measurements of length and width were taken at three intervals: (a) prior to excision, (b) 1 min after removal and (c) after 24 to 48 h of 10% neutral buffered formalin fixation. The first and second measurements were taken by one of the three surgeons with the first being performed prior to infiltration with local anesthetic, and the second being performed 1 min after removal of the specimen from the body. The third measurement was taken at the dermatopathology laboratory by a single senior laboratory technician. All measurements were taken with standard millimeter rulers. Difference in length, width and percent change were calculated for each specimen. Additionally, the specimens with the most shrinkage (six in all shrinking > 35%) and those with the least shrinkage (nine in all shrinking < 7%) were further evaluated with elastic stain (Verhoeff von Giesen) to determine if the amount and depth of elastin damage could account for the differences in the shrinkage. Independent readings were performed by two dermatopathologists to determine width of the scar as a percent of the specimen size, abnormalities or damage to oxytalan and eulanin fibers, and overall solar elastosis. All analyses were conducted on SAS version 9.1. Wright State University Institutional Review Board approval was obtained prior to the onset of the study.

Results

A total of 97 excisional specimens were included in the study, 42 from the trunk, 23 from the head and 32 from the limbs. Simultaneous 95% confidence intervals for mean shrinkage were $20.66\% \pm 2.15\%$ for length and $11.79\% \pm 2.35\%$ for width [Range of shrinkage: 0 to 41.18% for length and -18.75% (indication expansion) to 37.50% for width. The majority of tissue shrinkage occurred immediately after excision and prior to fixation. Interestingly, the average specimen shrank in length and width immediately after excision and then re-expanded slightly with formalin fixation.

Using linear regression with proportional shrinkage of area as the outcome, and patient age, site and gender as predictors, the model was significant ($p < 0.0001$) and accounted for about 25% of the variance between outcome and predictors (the R-square). Significant results were as follows: patient age is strongly significant in determining the proportional amount of shrinkage ($p < 0.0001$). For each year of age, the amount of shrinkage decreases by 0.3%. Site is marginally significant ($p = 0.0764$) when comparing head/neck specimens to the trunk. Compared to the head/neck, the proportional amount of shrinkage is approximately 5% more on the trunk.

Elastic stains were performed on the specimens with the most shrinkage (six in all shrinking > 35%, average age 40 years, range 8 to 56 years; one from the limbs, two from the trunk and three from head/neck sites) and those with the least shrinkage (nine in all shrinking < 7%, average age 66 years, range 51 to 75 years; five from the limbs, one from the trunk and three from head/neck sites). The width of the biopsy scar as a percent of specimen size was estimated as $\leq 25\%$ in ten, 50% in three and 75% in two. Scar width did not predictively correlate with shrinkage. Most scars were fairly superficial.

Damage to oxytalan and eulanin fibers as well as overall solar elastosis was evaluated to determine if a gradation of solar damage with respect to depth of solar injury could be assessed. Specimens prepared with elastic stain were graded using a rating of from 1 (normal) to 5 (severe damage) for each of the 15 specimens. Thickening of fibers, fragmentation and clumping were used as indicators of damage. Of the six specimens with the most significant increase in shrinkage, two had oxytalan damage rated as three or greater. Of the nine specimens with the least shrinkage, seven had oxytalan damage rated as three or greater. Overall damage scores for both oxytalan and eulanin fibers paralleled each other and the total solar elastosis score.

Overall solar elastosis was evaluated on a similar rating system. Of the six specimens with the most shrinkage, none had moderate or severe solar elastosis. However, of the nine specimens showing a relative lack of shrinkage, five had moderate or severe solar elastosis (rated four or five on a five-point scale) (Figure 1 and 2).

Discussion

Prior studies of tissue shrinkage have looked at histology (benign vs. malignant tumor excised), patient

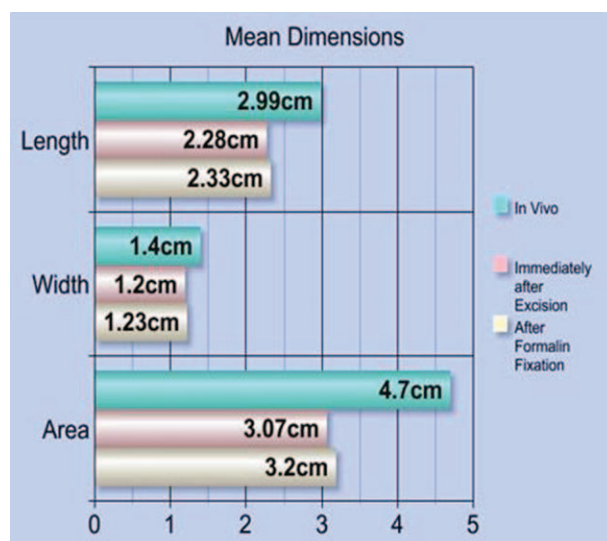


Fig. 1. Mean dimensions of specimens.

Shrinkage of cutaneous specimens

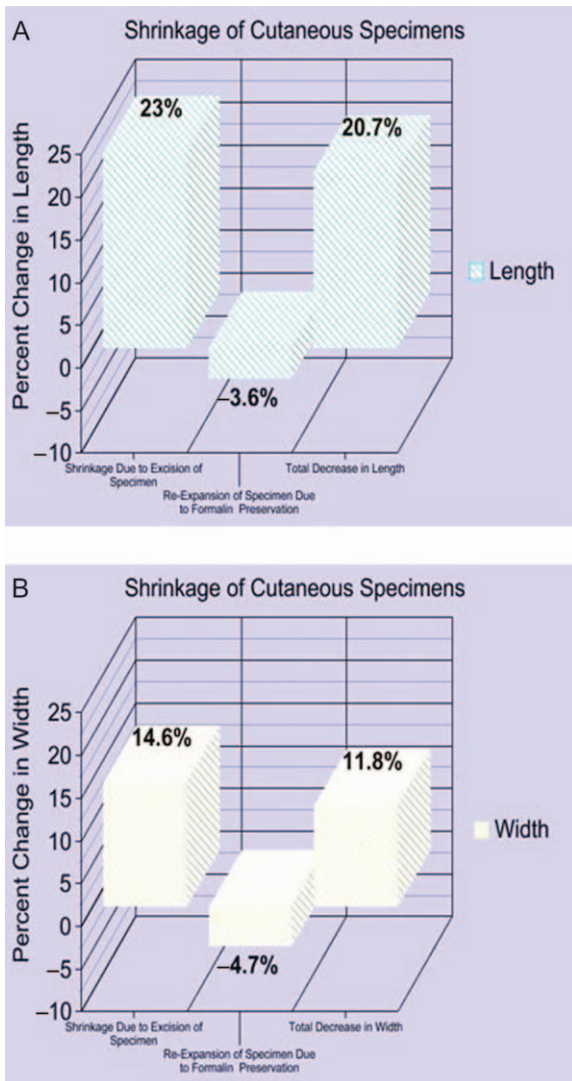


Fig. 2. Shrinkage of cutaneous specimens.

age, site, gender and formalin fixation prior to staining in determining variables that affect shrinkage during tissue processing. It has been reported that benign tumors shrink more than malignant tumors.⁸ In frozen sections from Mohs micrographic surgery specimens, shrinkage was statistically greater during processing in those above age 60 years, and tissue from the trunk and extremities showed significantly more shrinkage during processing than that from the head and neck.⁹ Gender has been determined to not affect shrinkage.^{8,9} In some studies, the use of formalin for preservation has been found to further increase the shrinkage seen in excised cutaneous specimens;⁸ however, others have concluded that formalin is non-contributory to shrinkage of cutaneous specimens, with the majority of shrinkage occurring at time of tissue removal from the body.¹⁰ Given these differences in outcomes, our study specifically evaluates excisional specimens from various

sites, ages and genders and determines when and to what degree shrinkage occurs during processing.

The findings of this study indicate that the majority of cutaneous tissue shrinkage post-excision is because of intrinsic contractile properties of the tissue itself, and not to fixation in formalin. The intrinsic contractile properties of the tissue are negatively affected by both the aging process and the solar damage as measured by solar elastosis.

Findings of the current study differ from prior cutaneous studies. This study found that for each year of age, the amount of shrinkage decreases by 0.3% ($p < 0.0001$). This decrease was relatively constant across age groups in contrast to Golomb et al. who found distinct cutoffs at age 50 and 60 that affected shrinkage. In Golomb's study, melanoma specimens were evaluated, and age was specifically found to be contributory to shrinkage. They delineated that patients below 50 years had the greatest shrinkage and those above 60 years had the least. Using final specimen size to determine operative margins, they developed formulas that used different age adjustment factors for those < 50 , 50 to 59 and ≥ 60 years. In the Hudson-Peacock's study, there was no evidence that the tissue specimen shrinkage was dependent on age, sex or site. However, a correlative finding related to age and tissue contraction (recoil) was noted in that for limb and trunk sites, wounds were larger than planned excision size in all patients and that this effect was greater in younger patients.⁸ Gardner et al.⁹ looked at Mohs micrographic specimens and found that shrinkage was statistically greater in those above age 60 years and in tissue from the trunk and extremities; however, this shrinkage was determined by comparing size after excision to that after histologic processing.

In the current study, tissue specimens shrank in length and width immediately after excision and then on average, re-expanded slightly with formalin fixation. These findings parallel those found in esophageal carcinoma where tumor shrinkage rate was 83.59% after resection but only 80.92% after formalin fixation.¹ In contrast, Hudson-Peacock et al.⁸ previously noted that specimens shrank by 22% post-excision with a further 11% reduction in size after fixation. Golomb et al.¹⁰ also found that the majority of tissue shrinkage occurred prior to formalin fixation.

Site of excision was marginally significant ($p = 0.0764$) when comparing head/neck with trunk with approximately 5% more shrinkage on the trunk. As previously mentioned, Hudson-Peacock et al. were only able to show greater wound retraction on the trunk, a finding that could be attributed to greater tension vectors on the surrounding skin. This study confirms that truncal skin exhibited relatively greater intrinsic shrinkage than skin from the head and neck.

Relatively sun protected truncal skin and specimens of younger patients exhibited greater shrinkage, suggesting that normal aging and solar damage lead to decreased contractility. To show this, elastin studies were performed. Damage to subsets of elastic fibers (oxytalan and eulanin) paralleled overall solar elastotic damage and showed roughly equivalent solar damage occurring at all depths of the dermis. The degree of solar elastosis correlated positively to lack of shrinkage, confirming that solar damage led to decreased contractility. Specimens from younger patients showed a greater amount of elastin on average, a finding reflecting the normal aging process.¹²

In summary, we conclude that post-excision tissue shrinkage is approximately 21% for length, 12% for width and 16% for area. It is primarily because of the intrinsic contractile properties of the skin. These contractile properties are diminished with patient aging and solar damage, both of which decrease the viable elastin in the skin. Formalin fixation does not cause tissue shrinkage. The clinician and dermatopathologist must be cognizant of the expected shrinkage of submitted specimens for settling discrepancies within the medical record.

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